

## CLAIMS

1. A method for generating an antagonist of the pro-survival Bcl-2 family said method comprising selecting a scaffold BH3-only protein structure with residue positions defining an amphipathic  $\alpha$ -helix formed by the BH3 domain; selecting one or more residue positions associated with a promiscuous binding phenotype of a BH3-only protein; substituting amino acid residues conferring a promiscuous phenotype for amino acids or their chemical analogs which confer a restrictive binding pattern to a Bcl-2 protein; and analyzing the interaction of each substitution for an ability to induce a more restrictive spectrum of binding to a Bcl-2 protein.
2. The method of Claim 1 wherein the Bcl-2 antagonist is specific for one or more of Bcl-2, Bcl-x<sub>L</sub>, Bcl-w, Mcl or A1.
3. The method of Claim 1 or 2 wherein the Bcl-2 antagonist is based on the structure of a molecule selected from the list consisting of Noxa, Bim, Puma, Bmf, Bad, Bik, Hrk and Bid.
4. The method of Claim 1 wherein the Bcl-2 antagonist inhibits a pro-survival protein on a cancer cell.
5. The method of Claim 4 wherein the cancer cell is selected from the list consisting of ABL1 protooncogene, AIDS Related Cancers, Acoustic Neuroma, Acute Lymphocytic Leukaemia, Acute Myeloid Leukaemia, Adenocystic carcinoma, Adrenocortical Cancer, Agnogenic myeloid metaplasia, Alopecia, Alveolar soft-part sarcoma, Anal cancer, Angiosarcoma, Aplastic Anaemia, Astrocytoma, Ataxia-telangiectasia, Basal Cell Carcinoma (Skin), Bladder Cancer, Bone Cancers, Bowel cancer, Brain Stem Glioma, Brain and CNS Tumors, Breast Cancer, CNS Tumors, Carcinoid Tumors, Cervical Cancer, Childhood Brain Tumors, Childhood Cancer, Childhood Leukaemia, Childhood Soft Tissue Sarcoma, Chondrosarcoma, Choriocarcinoma, Chronic Lymphocytic Leukaemia, Chronic Myeloid Leukaemia, Colorectal Cancers, Cutaneous T-Cell Lymphoma,

Dermatofibrosarcoma-protuberans, Desmoplastic-Small-Round-Cell-Tumor, Ductal Carcinoma, Endocrine Cancers, Endometrial Cancer, Ependymoma, Esophageal Cancer, Ewing's Sarcoma, Extra-Hepatic Bile Duct Cancer, Eye Cancer, Eye: Melanoma, Retinoblastoma, Fallopian Tube cancer, Fanconi Anaemia, Fibrosarcoma, Gall Bladder Cancer, Gastric Cancer, Gastrointestinal Cancers, Gastrointestinal-Carcinoid-Tumor, Genitourinary Cancers, Germ Cell Tumors, Gestational-Trophoblastic-Disease, Glioma, Gynaecological Cancers, Haematological Malignancies, Hairy Cell Leukaemia, Head and Neck Cancer, Hepatocellular Cancer, Hereditary Breast Cancer, Histiocytosis, Hodgkin's Disease, Human Papillomavirus, Hydatidiform mole, Hypercalcemia, Hypopharynx Cancer, IntraOcular Melanoma, Islet cell cancer, Kaposi's sarcoma, Kidney Cancer, Langerhan's-Cell-Histiocytosis, Laryngeal Cancer, Leiomyosarcoma, Leukaemia, Li-Fraumeni Syndrome, Lip Cancer, Liposarcoma, Liver Cancer, Lung Cancer, Lymphedema, Lymphoma, Hodgkin's Lymphoma, Non-Hodgkin's Lymphoma, Male Breast Cancer, Malignant-Rhabdoid-Tumor-of-Kidney, Medulloblastoma, Melanoma, Merkel Cell Cancer, Mesothelioma, Metastatic Cancer, Mouth Cancer, Multiple Endocrine Neoplasia, Mycosis Fungoides, Myelodysplastic Syndromes, Myeloma, Myeloproliferative Disorders, Nasal Cancer, Nasopharyngeal Cancer, Nephroblastoma, Neuroblastoma, Neurofibromatosis, Nijmegen Breakage Syndrome, Non-Melanoma Skin Cancer, Non-Small-Cell-Lung-Cancer-(NSCLC), Ocular Cancers, Oesophageal Cancer, Oral cavity Cancer, Oropharynx Cancer, Osteosarcoma, Ostomy Ovarian Cancer, Pancreas Cancer, Paranasal Cancer, Parathyroid Cancer, Parotid Gland Cancer, Penile Cancer, Peripheral-Neuroectodermal-Tumors, Pituitary Cancer, Polycythemia vera, Prostate Cancer, Rare-cancers-and-associated-disorders, Renal Cell Carcinoma, Retinoblastoma, Rhabdomyosarcoma, Rothmund-Thomson Syndrome, Salivary Gland Cancer, Sarcoma, Schwannoma, Sezary syndrome, Skin Cancer, Small Cell Lung Cancer (SCLC), Small Intestine Cancer, Soft Tissue Sarcoma, Spinal Cord Tumors, Squamous-Cell-Carcinoma-(skin), Stomach Cancer, Synovial sarcoma, Testicular Cancer, Thymus Cancer, Thyroid Cancer, Transitional-Cell-Cancer-(bladder), Transitional-Cell-Cancer-(renal-pelvis/-ureter), Trophoblastic Cancer, Urethral Cancer, Urinary System Cancer, Uroplakins, Uterine sarcoma, Uterus Cancer, Vaginal Cancer, Vulva Cancer, Waldenstrom's-Macroglobulinemia, Wilms' Tumor.

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6. The method of any one of Claims 1 to 5 wherein the Bcl-2 antagonist inhibits a prosurvival protein in a mammal.
7. The method of Claim 6 wherein the mammal is a human.
8. A method for generating or selecting an antagonist of the pro-survival Bcl-2 protein family said method comprising selecting a restrictive BH3-only protein as a scaffold protein, determining the conformation of the scaffold conferring the restrictive phenotype and generating or screening for a chemical compound which mimics said scaffold and/or conformational part conferring a restrictive spectrum of binding to a Bcl-2 protein.
9. Use of a promiscuous BH3-only protein as an amino acid residue substitute matrix in the generation or selection of substitute variants conferring a restrictive binding phenotype to said BH3-only protein or its chemical or conformational equivalent.
10. A computational method for designing an antagonist of the pro-survival Bcl-2 protein family based on a scaffold BH3-only protein with residue positions conferring a restrictive phenotype the method comprising selecting a collection of promiscuous BH3-only proteins; providing a sequence alignment of these proteins and comparing same to a restrictive BH3-only protein; generating a frequency of occurrence for individual amino acids in one or a plurality of positions with said alignments conferring promiscuity or restrictivity with respect to binding to Bcl-2 proteins; creating a scoring function selected from charge, size, conformation, solubility, polarity, hydrophobicity, hydrophilicity and contribution to tertiary structure using said frequencies; using said scoring function and at least one additional scoring function to generate a set of optimized protein sequences or their conformational equivalents and generating or selecting a compound or protein having a restrictive binding phenotype to a Bcl-2 protein.

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11. The method of Claim 10 wherein the scoring function is selected from (a) the number and position of acidic residues; or (b) the number and position of basic residues; or (c) the number and position of polar residues; or (d) the number and position of non-polar residues; or (e) the number and position of charged residues; or (f) the number and position of uncharged residues; or (g) the number and positions of hydrophilic residues; or (h) the number and position of hydrophobic residues; or (i) the levels of residues; or (j) the solubility levels of residues; or (k) the size of residues; or (l) the contribution to tertiary structure the residue makes in the BH3-only protein.

12. A computer program product for determining the structure of an agent to induce apoptosis in a cell, said product comprising:

(1) code that receives as input scoring function (SF) for at least two features associated with said BH3-only or Bcl-2 proteins, wherein said features are selected from, *inter alia*,:

- (m) the number and position of acidic residues;
- (n) the number and position of basic residues;
- (o) the number and position of polar residues;
- (p) the number and position of non-polar residues;
- (q) the number and position of charged residues;
- (r) the number and position of uncharged residues;
- (s) the number and position of hydrophilic residues;
- (t) the number and position of hydrophobic residues;
- (u) the levels of residues;
- (v) the solubility levels of residues;
- (w) the size of residues;
- (x) the contribution to tertiary structure the residue makes in the BH3-only protein

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- (2) code that adds said SF to provide a sum corresponding to a  $P_v$  for BH3-only proteins; and
- (3) a computer readable medium that stores the codes.

13. A computer for assessing the likely usefulness of a BH3-only protein or chemical equivalent to induce apoptosis in a cell, wherein said computer comprises:

(1) a machine-readable data storage medium comprising a data storage material encoded with machine-readable data, wherein said machine-readable data comprise  $I_{vs}$  for at least two features associated with said BH3-only or Bcl-2 proteins, wherein said features are selected from, *inter alia*,:

- (m) the number and position of acidic residues;
- (n) the number and position of basic residues;
- (o) the number and position of polar residues;
- (p) the number and position of non-polar residues;
- (q) the number and position of charged residues;
- (r) the number and position of uncharged residues;
- (s) the number and position of hydrophillic residues;
- (t) the number and position of hydrophobic residues;
- (u) the levels of residues;
- (v) the solubility levels of residues;
- (w) the size of residues;
- (x) the contribution to tertiary structure the residue makes in the BH3-only protein

(2) a working memory for storing instructions for processing said machine-readable data;

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(3) a central-processing unit coupled to said working memory and to said machine-readable data storage medium, for processing said machine readable data to provide a sum of said SF corresponding to a  $P_v$  for said compound(s); and

(4) an output hardware coupled to said central processing unit, for receiving said  $P_v$ .

14. A method of preventing or reducing cancer in a subject said method comprising administering to said subject an effective amount of an antagonist of a Bcl-2 protein for a time and under conditions sufficient to prevent or decrease cancer.

15. The method of Claim 14 wherein the antagonist is combined with one or more pharmaceutically acceptable carriers and/or diluents to form a pharmacological composition.

16. The method of Claim 14 or 15 wherein administration of the antagonist is administered by a respiratoral, intratracheal, nasopharyngeal, intravenous, intraperitoneal, subcutaneous, intracranial, intradermal, intramuscular, intraocular, intrathecal, intracerebral, intranasal, infusion, oral, rectal, patch or implant rates.

17. A composition comprising an antagonist of the pro-survival Bcl-2 protein family said antagonist generated by the method of a scaffold BH3-only protein structure with residue positions defining an amphipathic  $\alpha$ -helix formed by the BH3 domain; selecting one or more residue positions associated with a promiscuous binding phenotype of a BH3-only protein; substituting amino acid residues conferring a promiscuous phenotype for amino acids or their chemical analogs which confer a restrictive binding pattern to a Bcl-2 protein; and analyzing the interaction of each substitution for an ability to induce a more restrictive spectrum of binding to a Bcl-2 protein, said composition further comprising one or more pharmaceutically acceptable carriers and/or diluents.

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18. Use of an antagonist of the pro-survival Bcl-2 protein family said antagonist generated by the method comprising a scaffold BH3-only protein structure with residue positions defining an amphipathic  $\alpha$ -helix formed by the BH3 domain; selecting one or more residue positions associated with a promiscuous binding phenotype of a BH3-only protein; substituting amino acid residues conferring a promiscuous phenotype for amino acids or their chemical analogs which confer a restrictive binding pattern to a Bcl-2 protein; and analyzing the interaction of each substitution for an ability to induce a more restrictive spectrum of binding to a Bcl-2 protein in the manufacture of a medicament for the treatment of cancer.